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The sensitivity of the child to sex steroids: possible impact of exogenous estrogens

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The current trends of increasing incidences of testis, breast and prostate cancers are poorly understood, although it is assumed that sex hormones play a role. Disrupted sex hormone action is also believed to be involved in the increased occurrence of genital abnormalities among newborn boys and precocious puberty in girls. In this article, recent literature on sex steroid levels and their physiological roles during childhood is reviewed. It is concluded that (i) circulating levels of estradiol in prepubertal children are lower than originally claimed; (ii) children are extremely sensitive to estradiol and may respond with increased growth and/or breast development even at serum levels below the current detection limits; (iii) no threshold has been established, below which no hormonal effects can be seen in children exposed to exogenous steroids or endocrine disruptors; (iv) changes in hormone levels during fetal and prepubertal development may have severe effects in adult life and (v) the daily production rates of sex steroids in children estimated by the Food and Drug Administration in 1999 and still used in risk assessments are highly overestimated and should be revised. Because no lower threshold for estrogenic action has been established, caution should be taken to avoid unnecessary exposure of fetuses and children to exogenous sex steroids and endocrine disruptors, even at very low levels.

Key words: anabolic growth promoters/endocrine disruptor/estradiol/prepubertal children/threshold level

Introduction

During the past decades, there have been increasing indications that exogenous factors may influence the endogenous hormone balance. These include secular trends in hormone-dependent conditions and diseases such as earlier pubertal development (Herman-Giddens *et al*., 1997; Sun *et al*., 2002; Wu *et al*., 2002), increasing incidence of precocious puberty (PP) (Teilmann *et al*., 2005) and hormone-related cancers like testicular (Huyghe *et al*., 2003), breast (Parkin *et al*., 2005) and prostate cancers (Black *et al*., 1997). Furthermore, similar trends may apply to other disorders of the male reproductive tract, e.g. cryptorchidism (Boisen *et al*., 2004), hypospadias (Paulozzi, 1999; Toppari *et al*., 2001; Boisen *et al*., 2005) and poor semen quality (Swan *et al*., 2000; Jouannet *et al*., 2001; Jorgensen *et al*., 2002). These findings have caused raising concerns of the possible impact of endocrine disruptors on human health and support the hypothesis that humans may be exposed to increasing exogenous influences on the normal endogenous hormone activity.

Because the fetal and childhood periods are considered the most vulnerable for exogenous sex hormone activity, special concern has been directed against fetal and prepubertal exposure. In infancy, the hypothalamic-pituitary-gonadal (HPG) axis is active during the first months of postnatal life, the so-called mini-puberty, but is thereafter believed to be relatively quiescent until it is reactivated as the child enters puberty (Forest *et al*., 1974; Andersson *et al*., 1998; Chellakooty *et al*., 2003). The onset of puberty is traditionally characterized by an increased pulsatile release of hypothalamic GnRH resulting in a pulsatile gonadotropin secretion. However, more sensitive gonadotropin assays have now shown that the pulsatile nature of the LH and FSH secretion is present already before puberty in the supposed quiescent period of the HPG axis (Dunkel *et al*., 1992; Apter *et al*., 1993; Mitamura *et al*., 1999; Mitamura *et al*., 2000). Equally, new highly sensitive assays have documented that very low concentrations of estradiol are present in both boys and girls before puberty (Klein *et al*., 1994; Paris *et al*., 2002). This suggests that the HPG axis is functionally active and plays a biological role during childhood.

In this article, we review the current literature on sex steroid activity and its physiological importance in children before puberty. We will focus on both endogenous activity and the possible impact

of exogenous factors such as endocrine disruptors and natural and synthetic hormones from food products.

The pitutary-gonadal hormone axis in prepubertal children

The first reports on estradiol concentrations in serum from prepubertal children using radioimmunoassays were published in the 1970s (Jenner *et al*., 1972; Bidlingmaier *et al*., 1973; Angsusingha *et al*., 1974; Baker *et al*., 1976; Ducharme *et al*., 1976). According to these reports, the levels of estradiol in prepubertal boys and girls were apparently similar, ranging from 22 to 41 pmol/l. However, the plasma levels of estradiol in these studies appeared in most cases to be below or close to the detection limit of the assays. Comparisons of the early radioimmunoassays with today's more sensitive assays have indicated that the variation of the estradiol measurements was most pronounced for low concentrations with a tendency towards overestimating the estradiol concentrations giving false high serum levels in children as a result (McShane *et al*., 1996; Ikegami *et al*., 2001; Juul, 2001; Dorgan *et al*., 2002; Nelson *et al*., 2004; Wang *et al*., 2005).

In 1994, Klein and coworkers developed an ultrasensitive recombinant yeast bioassay (RCBA) and reported almost 100-fold lower estradiol concentration levels in prepubertal children compared with previously reported concentrations. The sensitivity of this bioassay was 0.07–0.7 pmol/l, and not only did the levels of estradiol measured in prepubertal children appear to be significantly lower than ever reported, but significantly lower estradiol levels (approximately eight-fold) were observed in prepubertal boys compared with those in prepubertal girls (mean concentration 0.3 and 2.2 pmol/l, respectively) (Klein *et al*., 1994; Larmore *et al*., 2002). Equally, recent studies of childhood levels of estradiol measured by radioimmunoassays with improved sensitivity have shown lower estradiol concentrations in boys as compared with girls of the same age (Norjavaara *et al*., 1996; Albertsson-Wikland *et al*., 1997; Ikegami *et al*., 2001). The difference between boys and girls was subsequently confirmed by another RCBA using human cells with a sensitivity of 3.7 pmol/l (Paris *et al*., 2002). However, the mean concentrations of estrogenic activity (estradiol equivalence) were estimated to be higher than the levels found by Klein *et al*. (1994) (corresponding in prepubertal girls to 12.9 pmol/l of estradiol and in prepubertal boys to 5.3 pmol/l of estradiol). Important differences between the two RCBAs, such as the use of different cell types and the presence or absence of ether extraction before measurement, might contribute to the major discrepancies in sensitivity and levels measured. Thus, although the levels of estradiol in prepubertal children over the last decade have been shown to be much lower than those previously reported, the true levels remain to be confirmed.

Equal to the problems of measuring low concentrations of sex steroids, results on gonadotropins in prepubertal children have been contradicting. In contrast to previous concepts, the use of improved assays has demonstrated that LH and FSH in both girls and boys are secreted in a pulsatile pattern years before the onset of puberty with a dramatic increase especially in LH pulsatility from prepuberty to pubertal onset (Dunkel *et al*., 1992; Apter *et al*., 1993; Mitamura *et al*., 1999, 2000). Corresponding to the rhythmicity of gonadotropin secretion in prepubertal children, estradiol and testosterone are secreted with peaks during the early morning and nadir in the late evening (Albertsson-Wikland *et al*., 1997; Ankarberg and Norjavaara, 1999; Mitamura *et al*., 1999; Mitamura *et al*., 2000; Ankarberg-Lindgren and Norjavaara, 2004). Some studies only found the rhythmicity of estradiol in late prepubertal girls (Norjavaara *et al*., 1996; Ankarberg and Norjavaara, 1999; Mitamura *et al*., 2000) and early pubertal boys (Dunkel *et al*., 1992; Albertsson-Wikland *et al*., 1997; Mitamura *et al*., 1999; Ankarberg-Lindgren and Norjavaara, 2004). This might, however, be due to the lack of assay sensitivity, and the diurnal rhythm may exist at extremely low levels in girls and boys before puberty, as has been indicated by Klein *et al*. (1998). The pulsatile nature of circulating concentrations of estradiol and testosterone in prepubertal girls and boys is an important factor to take into consideration in the evaluation of these hormones, because the determination of testosterone and estradiol in single blood sample may not be representative of the 24 h secretion.

Children are highly sensitive to sex steroid actions

Estrogen and androgen receptors are expressed in sex steroid sensitive tissues throughout childhood, and prepubertal children are therefore highly responsive to sex steroid actions. Because the prepubertal child's normal endogenous levels of sex steroids are very low, even a small variation would account for a major change in the total activity of the involved hormone, which is reflected in phenotypic effects in the child.

Palpable breast tissue is a common transient physiological condition in newborn infants, mainly thought to be related to exposure to maternal hormones *in utero* or through breastfeeding. In a study of 1126 3-month old children, serum levels of estradiol were significantly higher in the girls compared with those in the boys. Furthermore, a significant correlation between breast size and endogenous estradiol levels was observed among the girls, suggesting a sensitivity of breast tissue to sex steroids early in life (Schmidt *et al*., 2002). Accordingly, palpable breast tissue was significantly more frequent and pronounced in girls than in boys (Schmidt *et al*., 2002). The responsiveness of breast tissue to estrogen seems to be preserved also later in childhood, as the development of premature thelarche, defined by isolated breast development in a girl before the age of 8 years with no other clinical signs of sexual maturation, has been correlated to elevated estradiol levels (Klein *et al*., 1999).

Another example of an estrogen-sensitive tissue is bone. Observations in patients lacking estrogen actions because of estrogen receptor mutations (Smith *et al*., 1994) or aromatase deficiency (Conte *et al*., 1994; Morishima *et al*., 1995; Carani *et al*., 1997; Mullis *et al*., 1997) have indicated the importance of estradiol in epiphyseal maturation, normal skeleton proportions and bone mineralization in both sexes. Historically, estrogens have been regarded as growth repressive because of their role in the closure of epiphyses in puberty. However, subsequently, it has been shown that estradiol has a biphasic effect on epiphyseal growth, with the stimulation of linear growth at low concentrations and closure of the epiphyseal plates and cessation of linear growth at higher concentrations. The pubertal increase in growth velocity associated with increased growth hormone (GH) secretion has traditionally been attributed to testicular androgen secretion in boys and to estrogens or adrenal androgen secretion in girls. It has, however, been established that androgens influence the GH axis

only after aromatization into estrogens (Veldhuis *et al*., 1997). Thus, estradiol is probably the principal hormone stimulating the pubertal growth spurt in boys as well as girls.

The effect of physiological concentrations of estradiol on growth during childhood was exemplified in a study of girls with central PP undergoing GnRH agonist (GnRHa) therapy (Lampit *et al*., 2002). Continuous GnRHa therapy suppresses gonadotropin secretion and thereby the gonadal secretion of steroids. It has been suggested that GnRH therapy may suppress estradiol serum concentrations to subnormal prepubertal levels and thereby compromise normal growth. To secure a normal growth velocity, some girls in this study were substituted with a mini-dose of estrogen (8 µg of conjugated equine estrogen) to reach normal prepubertal estradiol levels during GnRHa therapy. Growth velocity in girls treated with both GnRHa and estradiol substitution was maintained, whereas growth velocity in girls treated with GnRH alone decreased. The levels of serum estradiol in the estrogen-substituted girls were below the detection limit of 14 pmol/l both before and after supplementation with estrogen, and no change in sexual development or acceleration of bone maturation was seen in these girls. This suggests that a very low (immeasurable with the conventional radioimmunoassays used) increase in serum estradiol had a significant influence on the growth of the girls without influencing sexual maturation (Lampit *et al*., 2002). Equally, the physiological importance of low concentrations of estradiol on growth and maturation is illustrated in Figure 1. The observed low-dose effects of estradiol are in accordance with results from animal studies, suggesting that there is no lower threshold for estrogenic action: any dose may have an effect (Sheehan, 2006).

Secular trends in pubertal timing

The difference in estradiol levels between prepubertal girls and boys may help understand various physiological phenomena better, such as the difference in body composition, skeletal maturation and timing of puberty. Especially, the higher level of estradiol in girls may account for their earlier onset of puberty. In girls, the pubertal growth spurt is known to start soon after the onset of puberty, whereas in boys growth spurt happens about 1 year after pubertal onset, and ultimately the cessation of growth occurs earlier in girls than in boys.

Two recent epidemiological studies in the United States (PROS and NHANES III) highlighted an unexpected advance in sexual maturation in girls (Herman-Giddens *et al*., 1997; Sun *et al*., 2002; Wu *et al*., 2002). In these two studies, mean age at breast development was 8.87 and 9.48 years, respectively, in African-American girls and 9.96 and 10.38 years, respectively, in white American girls (Herman-Giddens *et al*., 1997; Sun *et al*., 2002; Wu *et al*., 2002). Compared with the previous studies of pubertal onset in American girls, in which age at breast development was estimated to be 10.8–11.2 years (Foster *et al*., 1977; Lee, 1980), breast development occurred significantly earlier. The accuracy of these findings has been highly debated because of limitations in the study design, and further studies on pubertal onset have been requested. Several studies in other countries, however, have indicated a similar trend towards earlier sexual maturation in girls, although to a lesser extent than observed in the United States (Lindgren, 1996; Fredriks *et al*., 2000; Muinich Keizer and Mul, 2001; Parent *et al*., 2003; Castellino *et al*., 2005), whereas other studies did not find any changes in the pubertal development (Engelhardt *et al*., 1995; De Simone *et al*., 2004; Juul *et al*., 2006).

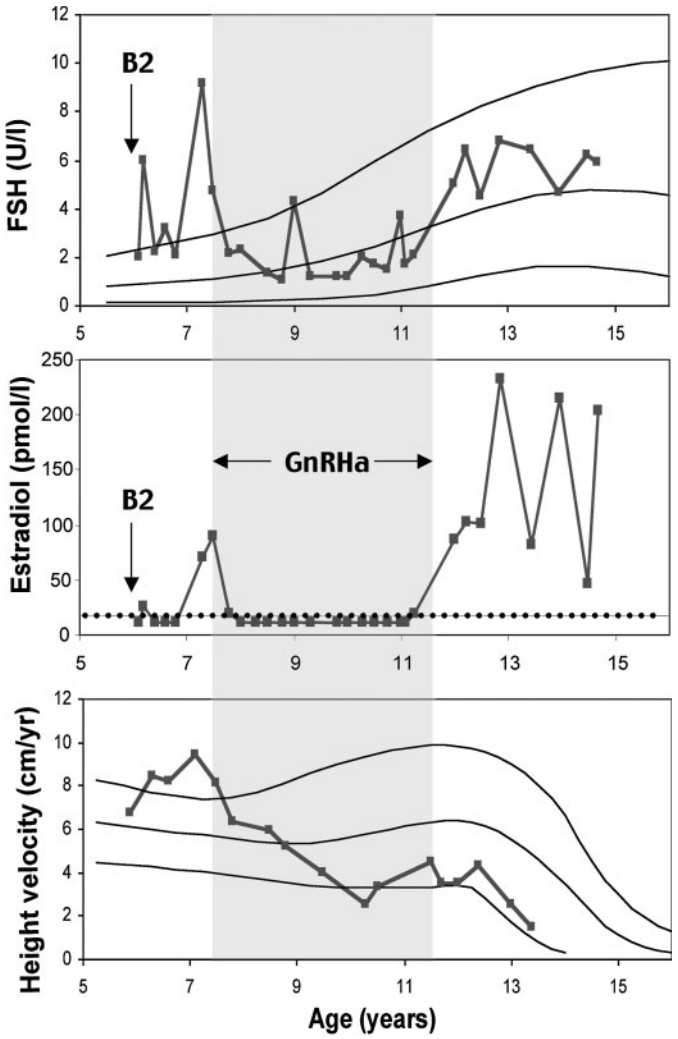

Figure 1. Time–course of biochemical and auxological changes in a girl with central precocious puberty before and during treatment with GnRH agonist (GnRHa) (indicated by shaded area). Individual serum levels of FSH (top panel) and estradiol (middle panel) are illustrated in comparison with normal ranges for healthy girls (Sehested *et al*., 2000). The concomitant growth changes are shown in the bottom panel compared with the normal ranges (Tanner *et al*., 1966). Increasing FSH levels stimulate ovarian estradiol production which in turn stimulates estrogen-responsive tissues. Note the presence of breast development (stage B2) and increased growth velocity approximately 1 year before estradiol levels become detectable (detection limit 18 pmol/l illustrated by dashed line in middle panel).

In contrast to girls, no secular trend in the male pubertal timing has been observed. Although adequate studies are lacking to cover this thoroughly, pubertal onset in European boys occurs much later than in boys in the United States: white American and African-American boys enter puberty at a mean age of 10.1 and 9.5 years, respectively (Herman-Giddens *et al*., 2001), whereas European boys enter puberty at an age between 11.2 and 11.8 years (Largo and Prader, 1983; Lindgren, 1996; Mul *et al*., 2001; De Simone *et al*., 2004; Juul *et al*., 2006).

In a register-based study on PP in Denmark from 1993 to 2001, the incidence of PP for girls aged 5–9 years was 8 per 10 000 and

for boys at the same age 1–2 per 10 000 (Teilmann *et al*., 2005). It can be speculated that the higher levels of estradiol in girls account for their higher incidence of PP than in boys, although the exact mechanisms of puberty onset remain obscure (Ojeda *et al*., 2006).

Puberty is traditionally considered precocious in a girl with breast development before the age of 8 years and in a boy with the occurrence of a testicular volume of more than 3 ml before the age of 9 years (Marshall and Tanner, 1969, 1970). However, because of the findings in the PROS study, this age limit has been taken into reconsideration in the United States, and it was suggested by the Lawson Wilkins Pediatric Endocrine Society to lower the age to 7 years in white girls and to 6 years in African-American girls. There was no evidence for changing the guidelines for evaluating boys (Kaplowitz and Oberfield, 1999). This recommendation has, however, been criticized. In a retrospective study, Midyett *et al.* (2003) found that 12.3% of 223 girls referred for true PP at age 7–8 (white girls) or 6–8 (African-American girls) had pathological explanations for their sexual precocity. Thus, lowering the age at which a girl should be evaluated might result in failure to identify cases with pathological conditions.

The timing and tempo of puberty is influenced by many factors such as genetics, nutritional status, ethnicity, environmental factors and geographic location [for review see (Parent *et al*., 2003)]. Because the prepubertal child is highly sensitive to changes in the endogenous hormonal milieu, which may influence pubertal maturation, these trends towards earlier maturation might be related to changes in the endogenous hormone balance and should as such not be ignored by lowering the age at which a child with early pubertal signs should undergo endocrine evaluation.

Biological impact of exposure to environmental xenoestrogens

In the industrial part of the world, the last generations have experienced a steady increase in several hormone-dependent adverse conditions and diseases. Several reports have pointed to a possible relation to increasing exposures to environmental pollutants and other endocrine disruptors (Toppari and Skakkebaek, 1998; Sultan *et al*., 2001; Rogan and Ragan, 2003).

Specific focus has been on compounds with direct estrogen-like action, i.e. the xenoestrogens (such as certain herbicides, pesticides, fungicides, plasticizers and polystyrenes). Because some of these compounds are used in food production and food packaging, one route of exposure that has caused concern is diet (e.g. pesticide residues on fruit and vegetables and food contaminated by compounds found in can lining and plastic wrapping), but the contribution from naturally occurring xenoestrogens such as phytoestrogens found in large concentrations in certain plants and mycoestrogens from fungi must also be considered. This is especially relevant in soy-based formulae containing high levels of phytoestrogens given to infants in a critical developmental period. However, many phytoestrogens are aromatase inhibitors at physiological concentrations, and they may therefore act to some extent as anti-estrogens rather than as estrogens (Almstrup *et al*., 2002). Many of the compounds originally identified as weak estrogens have subsequently also been shown to possess anti-androgenic properties, e.g. by reducing the testicular testosterone synthesis (Gray *et al*., 2006).

Several animal studies have shown that prenatal exposure to endocrine disruptors can produce adverse effects on male reproductive development similar to the trends observed among humans (Wood *et al*., 1991; Delemarre-van de Waal, 1993; Howdeshell *et al*., 1999; Fisher *et al*., 2003; Hotchkiss *et al*., 2004; Noriega *et al*., 2005; Vinggaard *et al*., 2005). Disturbed hormone balance during postnatal development can also lead to persistent adverse effects in the adult animal. Thus, in Rhesus monkeys, elevated levels of estradiol during gonadotropin-induced puberty caused inhibition of testicular growth and testosterone production, reduced number of Leydig cells and higher incidence of maldescensus of the testis compared to controls (Ramaswamy, 2005). Also exposure to the endocrine disruptor dibutyl phthalate during adolescent development in rabbits led to persistent disturbed endogenous testosterone production (Higuchi *et al*., 2003).

Exogenous exposure to natural and synthetic sex steroids

The natural estrogen estradiol is at least 10 000-fold more potent than most identified environmental xenoestrogens, and the dietary exposure (from e.g. meat, dairy products and eggs) to the natural sex steroids is therefore highly relevant in the discussion of the impact of estrogens on human development and health. Estradiol, progesterone and testosterone are substances that are naturally occurring in both humans and animals in identical molecular forms. Estradiol-17β is the most potent estrogenic substance, whereas the metabolites, estrol and estrone are less active (Gutendorf and Westendorf, 2001). Despite the lesser activity of these metabolites, they nevertheless contribute to the total bioactivity of estrogen in animals, as they can be present in high concentrations.

Cases of accidental exposures of children to estrogens have shown that children are sensitive to exogenous hormones (Beas *et al*., 1969; Gabrilove and Luria, 1978; Fara *et al*., 1979; Kimball *et al*., 1981; Edidin and Levitsky, 1982; Felner and White, 2000). Gynaecomastia was observed in three prepubertal boys because of indirect exposure to estrogen cream used by their postmenopausal mothers (Felner and White, 2000). These boys presented with gynaecomastia, advanced bone age and elevated estradiol levels, but 6 months after the removal of exposure, the gynaecomastia regressed and the levels of serum estradiol were immeasurable (Felner and White, 2000). An outbreak of gynaecomastia and elevated serum estradiol levels in boys and girls attending a school in Milan were reported. Poultry and beef from the school cafeteria were suspected as the sources, although this was never confirmed (Scaglioni *et al*., 1978; Fara *et al*., 1979).

Apart from these more extreme episodes of exposure, humans are commonly exposed to a wide variety of suspected endocrine disruptors. The exposure levels may be low and the potency of the compounds weak, and clear effects on endocrine function from such exposures are difficult to demonstrate. On the contrary, because of our modern way of living, the general population is exposed to many potential endocrine disruptors concomitantly. Both *in vitro* and *in vivo* studies have shown that the action of estrogenic compounds is additive (Rajapakse *et al*., 2002; Tinwell and Ashby, 2004), but little is known about the possible synergistic or additive effects of these compounds in humans (Toppari and Skakkebaek, 1998).

All edible tissues of animal origin contain estradiol and its metabolites in varying concentrations, depending on the kind of

tissue, species, gender, age and physiological stage of the animal. In addition to these naturally occurring endogenous hormones, the application of exogenous sex steroids and other hormones is licensed for use as active growth promoters in cattle production in several countries (e.g. the United States, Canada, Argentina, Australia and New Zealand), as their use improves the rate of weight gain or feed efficiency. This application of estradiol-17β, alone or in combination with other natural (testosterone or progesterone) or potent synthetic hormones (e.g. trenbolone, melengestrol acetate and Zeranol), results in increased amounts of residues of estrogens when compared with untreated animals (The Joint FAO/WHO Expert Committee on Food Additives, 1988; Maume *et al*., 2001; Stephany, 2001). The use of exogenous hormones as growth promoters has been forbidden since 1989 in all member states of the European Union (EU).

The US Food and Drug Administration's (FDA) guidelines suggest that the maximal secure intake of natural sex steroids equals 1% of the normal daily production rate of the relevant hormone of prepubertal children. In the case of estradiol, prepubertal boys synthesize the least, and the maximum acceptable daily intake (ADI) was therefore estimated to be 65 ng/day according to an estimated production rate of 6.5 μg/day (US Food and Drug Administration, 1999). Identical production rates were also given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1988). In recent reviews, however, the value of this estimated ADI has been questioned, as the daily production rates that it is based on are very doubtful (Andersson and Skakkebaek, 1999; Daxenberger *et al*., 2001; Maume *et al*., 2001; Partsch and Sippell, 2001). Daily production rates of hormones are calculated from the estimates of the metabolic clearance rate (MCR, the volume of serum that can be cleared of a given hormone during 24 h) and of the plasma concentrations of the given hormone according to the following equation:

Daily production rate (μg/day) = plasma concentration (μg/ml) × MCR (ml/day).

Because the concentrations of plasma estradiol in prepubertal children were highly overestimated in the measurements using early radioimmunoassays and because the MCR most probably was based on values obtained from adults, it has been suggested that the production rate of estradiol in prepubertal boys used by JECFA and FDA to determine the ADI might be 100–200 times higher than the actual production rate (Andersson and Skakkebaek, 1999). According to a revised calculation of the original production rate calculated by JECFA and FDA based on the estradiol measurements of Klein *et al*., the production rate of estradiol in prepubertal boys was reduced to a level between 0.04 and 0.1 μg/day (Andersson and Skakkebaek, 1999). The production rate of 0.04 μg/day correlates to an ADI of 400 pg/day (1% of 0.04 μg). Thus, although the actual serum concentrations of estradiol in prepubertal children remain to be unequivocally determined, all recent studies indicate that the physiological levels are significantly lower than those previously believed. Furthermore, the MCR for children is simply not available.

In a review on natural sex steroids in meat, the average concentrations of estradiol-17β in meat from untreated cattle was estimated to be 4.3 ng/500 g and from cattle treated with estradiol 20 ng/500 g (Daxenberger *et al*., 2001). Five hundred grams of meat was used according to the JECFA estimate of a theoretic daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat. Applying this to the revised threshold of 400 pg estradiol per day, this would be reached after the ingestion of 47 g untreated meat and already after ingestion of 10 g of treated meat (Daxenberger *et al*., 2001). On the basis of a standardized food intake, Daxenberger *et al*. (2001) estimated that the inclusion of meat from treated animals increased the daily intake of estrogen from food by 37.9%. Also the intake of pork, poultry, fish, eggs and dairy products contributes to the total dietary estradiol consumption. The concentrations of estrogens in dairy products depend on the physiological status of the lactating mammals. Modern dairy cows are often pregnant and may continue to lactate during the latter half of pregnancy when the estradiol concentration in blood and hence in milk increases significantly.

During the last decade, more adequate methods based on gas chromatography-mass spectrometry (GC-MS) have been developed to measure residues in food. Most studies on estradiol residues in meat include measurements of estradiol-17β and its major metabolites, estradiol-17α and estrone. A method to separate various classes of free estradiol and conjugates such as glucosidic and fatty acid ester forms was recently developed (Maume *et al*., 2001). Comparing the estrogen measurements using this method with previously reported measurements revealed a good correlation when analysing meat from untreated cattle. However, the increased levels of total estrogenicity in samples from treated animals turned out to be largely because of the lipoidal esters and not, as previously believed, because of free or classical conjugated forms of estradiol (Maume *et al*., 2001). The bioavailability and estrogenicity of these fatty acid esters of estradiol should be considered in the evaluation of human exposure. Thus, even though the concentration of estradiol may be within the range of untreated meat (including from pregnant heifers) (Stephany *et al*., 2004), the increased level of these metabolites in treated meat may potentially influence human health.

Equally, when evaluating the estrogenicity of synthetic growth promoters, the fraction of existing metabolites of the relevant compound has to be taken into account. In the case of Zeranol, a synthetic β-resorcylic acid lactone, there are six major forms that each can be metabolized into all the other forms. The expression of endogenous estrogen-related genes in human breast cancer MCF7 cells treated with increasing concentrations of Zeranol and its metabolites, diethylstilboestrol (DES), estradiol-17β, a phytoestrogen (genistein) and a putative endocrine disruptor, Bisphenol-A, was analysed to compare the estrogenic potency of these compounds (Leffers *et al*., 2001). Zeranol and its metabolites turned out to be as potent as DES and estradiol-17β, whereas genistein and Bisphenol-A were up to five and six orders of magnitude less potent estrogens, respectively. Significant and measurable changes in the MCF7 cell gene expression were observed at concentrations of 1 pmol/l of the three high-potency estrogens (Leffers *et al*., 2001). It has been shown that exposure to both low- and high-dose Zeranol treatment of rats caused significantly earlier vaginal opening, irregularity of estrous cycle (high frequency of prolonged estrus or prolonged diestrus) at 8–11 weeks of age and anovulatory ovaries (ovaries without newly formed corpora lutea) (Yuri *et al*., 2004). Because Zeranol has a significantly higher estrogenic potency than other potential endocrine disruptors, its presence in meat may constitute the major exposure to exogenous estrogens for consumers in countries where it is used for cattle production.

Other synthetic substances commonly used as growth promoters in cattle are the gestagen melengestrol acetate and the androgen trenbolone. Because these synthetic hormones have no legal application in humans, the knowledge on their effects in man is limited; however, melengestrol acetate and trenbolone bind to the human progesterone and androgen receptors with high affinities (Bauer *et al*., 2000).

The role of carrier proteins

The binding properties of endogenous and exogenous estrogens to serum proteins must also be considered. Because endogenous estradiol has a high affinity to hormone-binding proteins in serum [especially sex hormone-binding globulin (SHBG)] with about 98% bound, only a very small fraction of estradiol is in its free and bioavailable form. In contrast, most exogenous hormone-like chemicals including synthetic growth-promoting hormones exhibit limited or no binding to carrier proteins (Sheehan and Young, 1979; Shrimanker *et al*., 1985). Thus, the potency of synthetic hormones can be much larger than their actual concentrations suggest (Crain *et al*., 1998; Nagel *et al*., 1998). To evaluate the potential threat of growth promoters in meat production, one must take these factors into account.

Possible late effects of exposure to exogenous hormones in childhood

Sex hormone levels in early life may influence development of breast pathology such as breast cancer in adulthood (Ekbom *et al*., 1997). Epidemiologic evidence suggests that females exposed to elevated levels of maternal estradiol during fetal life have an increased risk of breast cancer in adult life (Mandybur *et al*., 1978; Ekbom *et al*., 1997; Swerdlow *et al*., 1997; Weiss *et al*., 1997; Halakivi-Clarke *et al*., 2000), whereas conditions with low levels of maternal estradiol (e.g. preeclampsia) have a protective effect on the female fetus against breast cancer in adulthood (Innes and Byers, 1999).

A study of the correlation between incidence rates for hormone-dependent cancers (breast, ovarian and corpus uteri cancers) and food intake revealed a strong association to the amount of animal-derived food consumed in the 40 countries studied. Especially, the consumption of milk and dairy products was of concern according to the fact that these products contain the highest concentration of estradiol (Ganmaa and Sato, 2005).

Moreover, recent evidence suggests that breast cancer originates from a pool of tissue stem cells (Smalley and Ashworth, 2003). Because estradiol levels during fetal development modulate the number of cells with stem cell potential, prenatal estradiol levels may play a significant role in the risk of breast cancer in the adult (Baik *et al*., 2005). Furthermore, a high birth weight, early age at peak growth, high stature at 14 years of age and low BMI at 14 years of age have been associated with a high risk of breast cancer in adulthood (Ahlgren *et al*., 2004); because even very low levels of estradiol significantly stimulate prepubertal growth, prepubertal exposure to low doses of exogenous estradiol may increase the risk of breast cancer later in life.

Some male reproductive health disorders may also have their origin in childhood. Recently, the existence of a testicular dysgenesis syndrome explaining some of the observed adverse trends in male reproductive health was suggested (Skakkebaek *et al*., 2001). According to this hypothesis, several male reproductive disorders such as testicular cancer, cryptorchidism, hypospadias and poor sperm quality may be because of a disturbance of the hormonal balance during the development of the reproductive organs. These adverse outcomes are thought to be related primarily to factors affecting fetal development. However, permanent effects on reproduction by exposures during childhood cannot be excluded as indicated by animal studies (Higuchi *et al*., 2003; Ramaswamy, 2005).

Conclusion

Low doses of sex steroids and synthetic molecules interfering with normal sex steroid action can exert significant effects in the fetus and the child, where a fine balance between the pituitary gland and the gonads exists. Recent data have shown that the endogenous (natural) levels of estradiol in children are significantly lower than those previously assumed. Exogenous contributions will therefore constitute a relatively higher proportion of the sex hormone activity in the immature child.

Exposure to environmental components with endocrine disrupting potencies is causing increasing concern. Intake through food is presumed to contribute significantly to the daily exposure, and the use of growth promoters in cattle production results in an increased total level of sex steroids in meat. A certain level of estradiol, testosterone and other sex steroids is (and has probably always been) present in food, e.g. meat, milk, and egg products. However, a systematic use of natural and synthetic steroids in meat production will undoubtedly expose the population in general to somewhat higher levels of sex hormones, although they often will be below detection limits (for the natural estradiol, testosterone and progesterone) or difficult to detect because of the lack of available methods for analysis (of the synthetic Zeranol, trenbolone and melengestrol acetate).

The question of possible effects of sex steroid exposure of children is extremely relevant, as we have been unable to find good evidence of a safe margin for exposure of children to sex hormones added to food products. Previous calculations seem to be based on flawed assumptions. With today's knowledge about the levels of sex steroids in prepubertal children, the FDA's estimated daily production rates of sex steroids in children are highly overestimated. We therefore recommend that the threshold for secure daily intake of sex hormones based on these estimates should be revised.

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